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Cultivation requirements and substrate degradation of the edible mushroom *Gymnopilus pampeanus*—A novel species for mushroom cultivation

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ABSTRACT

The production of new species of edible mushrooms is an innovative way to recycle agro-industrial wastes into food production. The genus *Gymnopilus* has a large number of xylophagous species being *Gymnopilus pampeanus* the only consumed species. The objective of this work is to determine the optimal condition needed to cultivate *G. pampeanus*, to evaluate its biological efficiency and to determine the biodegradation of substrate. *Populus* and *Eucalyptus* sawdust were used as substrates for production. We determined that light is necessary for a normal development of primordia. Strain ICFC 748/12 produces the highest biological efficiency on *Populus* sawdust reaching a mean of 70.67%. *G. pampeanus* has a strong capacity to degrade *Eucalyptus* and *Populus*. This mushroom has the ability to decompose cellulose and also to decay lignin, thus being white rot fungi. This is the first report of the cultivation of this species on lignocellulosic waste which turns it into a promising species for commercial production.

1. Introduction

The cultivation of edible mushrooms is a worldwide important commercial activity (Chang, 2000). Several aspects have contributed to the development of this activity: (a) the raw materials used are waste from agribusiness that have little commercial value and are easy to acquire (Rajarathnam and Bano, 1991), (b) the product obtained has a visually attractive appearance, pleasant taste and a high quality protein which provides much of the essential amino acids to the diet (Chang, 1991), (c) some species can be grown with relatively simple technology and low investment.

Worldwide commercial mushroom production has progressively improved during the last decade, but only a few genera of basidiomycetes (*Agaricus*, *Lentinula*, *Pleurotus*, *Auricularia*, *Volvariella*, *Flammulina*, *Tremella* and a few others) are industrially cultivated (Stamets, 1993). Many countries have abundance of agro-industrial wastes that can be used as substrates to produce mushrooms. In Argentina, *Populus* and *Eucalyptus* sawdust is abundant since these two types of woods are used to produce furniture or fruit wooden boxes. Most of the time, the sawdust is not reused and it is either piled up in fields for a slow degradation or even burnt. The use of these waste materials in a productive activity may help to improve the regional economies of the country. In recent years our laboratory developed techniques to adapt wild edible mushroom species to industrial crop, as *Lentinus tigrinus* (Lechner and Albertó, 2007), *Agrocybe cylindrica* (Uhart et al., 2008) and *Polyporus tenuiculus* (Omarini et al., 2009).

The genus *Gymnopilus* has a large number of xylophagous species that have not been deeply studied in Argentina yet. Twelve species of *Gymnopilus* were described for Argentina (Niveiro and Albertó, 2014). Wright and Albertó (2002) have described and illustrated two species which grow on wood of *Eucalyptus* spp. or *Pinus* spp.: *Gymnopilus chrysopellus* (Berk. et Curt.) Murr. and *Gspectabylis spectabylis* (Fr.) Singer var *pampeanus* (Speg.) Sing. (=*G. pampeanus* (Speg.) Singer). The latter in particular, which is consumed in Argentina and Uruguay (Albertó et al., 2010) is a species with a large pileus (10–20 cm diam.) that frequently produces clusters with many basidiomata. During the past years we have been collecting strains of *G. pampeanus* to test them in culture. The objectives of this work are to determine the optimal condition needed to cultivate the edible mushroom *G. pampeanus*, to evaluate its biological efficiency and to determine the biodegradation of spent substrates.

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2. Materials and methods

2.1. Strains used

Strains used are conserved in ICFC (IIB-INTECH Collection of Fungal Cultures, Laboratory of Mycology and Mushroom Cultivation, IIB-INTECH; Chascomús, Argentina), reference in the WDCM data base: 826.

G. pampeanus: ICFC 748/12, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 04-X-2011, *coll.* M. B. Colavolpe; *G. pampeanus*: ICFC 751/12, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 03-XI-2011, *coll.* M. B. Colavolpe; *G. pampeanus:* ICFC 776/13, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 25-IV-2012, *coll.* M. B. Colavolpe; *G. pampeanus*: ICFC 779/13, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 24-VII-2012, *coll.* M. B. Colavolpe; *G. pampeanus*: ICFC 47/99, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 25-III-1999, *coll.* E. Albertó.

2.2. Culture media and spawn production

Potato dextrose agar (PDA, Britania, Argentina, 39 g/l) culture medium was used for routine culture and storage purposes. Grain spawn was prepared in 750 ml glass jars filled with boiled oat grains and 1% w/w $CaCO_3$, then, they were sterilized for 2 h at 120 °C, cooled at room temperature and inoculated with an agar plug (1 cm diam.) of selected strain, cut from the advancing margin of a 5-d-old colony grown on PDA. Jars were incubated in the dark, at 25 °C, with periodical shaking during 30 days.

2.3. Optimal temperature for mycelium growth

Growth of mycelium was evaluated in PDA; plates were incubated at three temperatures: 20, 25 and 30 °C. 5 mm diameter cylinder of agar with actively growing mycelium was placed in the center of a Petri dish. Radial fungal growth was measured after one week. Three replicates of each strain were done; average growth and standard errors were calculated.

2.4. Determination of light necessity for basidiomata primordia induction

Three strains were selected for production assay: ICFC 47/99, ICFC 751/12 and 748/12. 25 × 40 cm polypropylene bags (30 μm thick) were filled with 100 g of *Eucalyptus* sawdust; moisture was adjusted to 70% of humidity using tap water and then sterilized at 120 °C, 1.2 psi for 2 h. Once bags, reached room temperature, they were inoculated with 5 g of spawn prepared with strain ICFC: 748/12. Then bags were moved to incubation room and maintained at 25 °C in the dark for 60 days. For this test we used an 80 × 40 × 30 cm fishbowl divided in to two equal compartments. Glass walls of one of them and the upper part were coated with opaque black polyethylene plastic film to avoid light from coming in and to keep it completely dark, the other compartment was covered with a transparent polyethylene plastic film (Fig. 1). A plastic tube was placed in both compartments to supply fresh air produced by a fishbowl aerator.

After spawn running, plastic bags were removed and four blocks were then placed in each compartment. Manual watering was daily carried out with a sprayer. The fishbowl was placed in the production room maintained at a temperature of 20 °C and with a photoperiod of 9 h light and 15 h dark. After 20 days primordia formation, form and color were evaluated. Color notations in parentheses are from The Online Auction Color Chart Company, 2010.

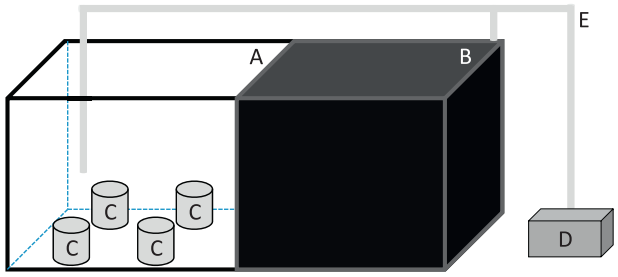

Fig. 1. Fishbowl design to evaluate light necessity for *G. pampeanus* primordia induction. (A) Light photoperiod compartment (9 h light, 15 h dark). (B) Dark compartment (without photoperiod). (C): Blocks of *G. pampeanus* growing on *Eucalyptus* sawdust. (D) Aerator. (E) Plastic tube for air supply.

2.5. Production assay

Strains ICFC 47/99, 748/12 and 751/12 were used in the production assay.

2.5.1. Substrate preparation

Eucalyptus sp. sawdust (ES) and *Populus* sp. sawdust (PS) were used as substrates, both pure with no supplementation. 25 × 45 cm polypropylene bags were filled with 300 g of dry substrate per bag; 1% of $CaCO_3$ powder was added. The moisture was adjusted to 70% using tap water; bags finally reached 1000 g. They were autoclaved for 2 h at 120 °C and 1.2 psi.

2.5.2. Inoculation and methodology of production

Bags were inoculated with spawn at 5% w/w in laminar flow. Spawn running was carried out in the incubation room at 25 °C in darkness during 75 days. After complete colonization of the substrate, plastic bags were removed and the colonized substrates, referred hereafter as production blocks, were transferred to production room (2.5 × 4.5 m). Temperature (18–20 °C), humidity levels (70–80%), ventilation, spray watering (5 min, 5 times per day) and photoperiod (9 h light/15 h dark) using a 20 W fluorescent light (400–700 nm) were automatically controlled to induce basidiomata formation.

2.5.3. Quality trait assessment

(1) Primordium initiation (days): time until appearance of primordium under fruiting conditions was registered. (2) Yield production: biological efficiency BE (%) was used to evaluate the yields (BE% = Mushroom wet weight g/Substrate dry weight g × 100). (3) Measures of basidiomata harvested: the mushrooms were measured and weighted; pileus diameter (cm), stipe length (cm) and stipe diameter (cm) were taken with a ruler (0.5 mm scale). The diameter of the stipe was measured 2 cm above the stipites base.

2.5.4. Statistical analyses

Prior to analysis, Kolmogorov–Smirnov test (with Lilliefors' correction) and Levene median test were applied to test data for normality and equal variation. Differences between mean values of treatments were analyzed with a two-way ANOVA analysis, followed by all pairwise multiple comparison procedures (post hoc testing). SPSS Statistics 17.0 program was used to detect Significant Differences ($P < 0.05$) between media treatments using the General linear Model. Six replicates per each treatment were made.

2.6. Biodegradation of substrates during cultivation process

The strain which produced the highest yields (ICFC: 748/12) was selected for biodegradation analysis in both substrates. The percentages of lignin, hemicelluose and cellulose were analyzed using

Table 1
Growth rate (cm/day) of *G. pampeanus* at three different temperatures.

Strains	Linear growth rate (cm/day)		
	20 °C	25 °C	30 °C
47/99	0.30 ± 0.14b	0.73 ± 0.11a	0.43 ± 0.04b
748/12	0.35 ± 0.07b	0.55 ± 0.07a	0.35 ± 0.06b
751/12	0.35 ± 0.21b	0.58 ± 0.11a	0.40 ± 0.00b
776/13	0.38 ± 0.25b	0.55 ± 0.21a	0.35 ± 0.00b
779/13	0.35 ± 0.21b	0.58 ± 0.18a	0.33 ± 0.04b

Means followed by the same letter in the same row are not significantly different according to Tukey's test.

the spent substrate at the end of production assay; control bags were evaluated at zero time (substrate without treatment). AOAC International methodology (AOAC International, 1990a,b) was used for this analysis. The dry matter content of each substrate at different stages was determined by drying samples to a constant mass in a 60 °C oven (Zhang et al., 2007). The fiber component of all substrates was determined using the detergent method (Goering and Van Soest, 1970) and both the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) were extracted. Lignin fractions were determined with sulphuric acid (72%), and the cellulose content was estimated directly from ADF–lignin. Hemicelluose was arithmetically calculated as NDF–ADF (Goering and Van Soest, 1970). All results were presented on a dry matter basis (%). Three replicates of each treatment were randomly selected for this evaluation.

3. Results

3.1. Determination of linear growth rates

The results showed that all strains of *G. pampeanus* had an optimal growth at 25 °C (Table 1). The growth was slower when the temperature was 20 or 30 °C. Strain ICFC 47/99 had the faster rate growth with 0.73 cm/day; the rest of the strains had a slower growth at 25 °C which varied from 0.55 to 0.58 cm/day.

3.2. Determination of light requirements for primordia induction

Some mushrooms such as *Pleurotus* spp. or *Lentinula edodes* require light for primordia formation (Stamets, 1993; Nakano et al., 2010). We observed that the development of basidioma primordia of *G. pampeanus* does not depend on light. In fact, we could observe primordia in both sides of the fishbowl in the light compartment as well as in the dark one (Fig. 2). Primordia obtained under light exposure were light brown colored (oac7) and longer than primordia obtained in the darkness (oac813). Thus, photoperiod is not necessary to induce the primordium formation. However, photoperiod is needed for fruiting body production. What is more, primordia that remained in darkness showed deformation and abnormal development meanwhile those which were exposed to photoperiod had normal growth (Fig. 2).

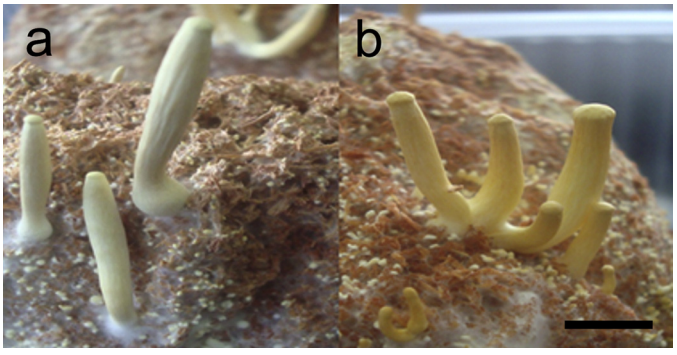

Fig. 2. Effect of light photoperiod on primordia growth of *G. pampeanus* (a) in darkness; (b) growing with photoperiod (9 light/15 darkness). Note that primordia obtained under light exposure are light brown colored (oac7) and longer. These will not develop without light (treatment: *Eucalyptus* sawdust and strain ICFC 751/12). Scale bar: 10 mm.

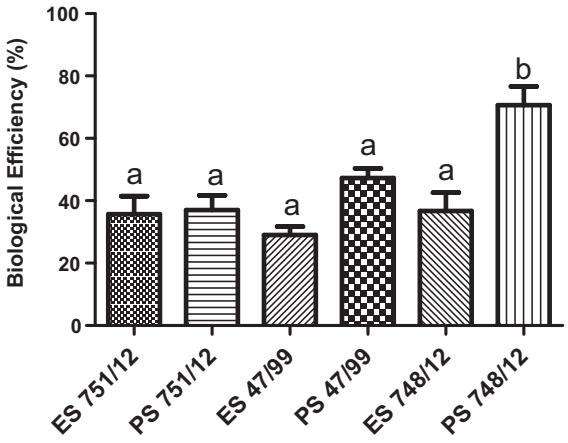

Fig. 3. Biological Efficiency (%) of *G. pampeanus* growing on sawdust. ES 751/12: *Eucalyptus* sawdust and strain ICFC 751/12; PS 751/12: *Populus* sawdust and strain ICFC 751/12; ES 47/99: *Eucalyptus* sawdust and strain ICFC 47/99; PS 47/99: *Populus* sawdust and strain ICFC 47/99; ES 748/12: *Eucalyptus* sawdust and strain ICFC 748/12; PS 748/12: *Populus* sawdust and strain ICFC 748/12. * Asterisk represents significant differences between treatments according to Tukey's test.

3.3. Production assay

Bags were incubated during 75 days, lapse which coincides with initiation of some primordia formation. After this period, bags were removed and the colonized substrates, referred hereafter as production blocks, were transferred to fruiting room. New primordia began to form after 11 days of moving bags to the production room. Mature fruiting bodies were produced after 22 days on PS (ICFC: 751/12) and after 30 days on ES (ICFC 47/99). Fruiting bodies were produced in most of the blocks; 100% of blocks made of PS produced basidiomata meanwhile 94.43% made of ES produced them. Treatments with strains ICFC 47/99 and 748/12 on ES also produced mushrooms in 100% of the blocks; only strain 751/12 produced fruit bodies in 83.33% of the blocks. The highest BE % was obtained with strain ICFC 748/12 on PS reaching a mean of 70.67% with significant difference ($P < 0.05$) in relation to the other treatments (Fig. 3). The strain ICFC 47/12 also produced higher yield on PS (47.33%) and lower on ES (29.04%). Strain 751/12 produced similar values in both substrates: 37.00% on ES (Fig. 4) and 35.72% on PS.

Fig. 4. Basidiomata and primordia of *G. pampeanus* obtained on *Eucalyptus* sawdust (strain ICFC 751/12). Scale bar: 3 cm.

Table 2
Basidiomata weight, pileus diameter, stipe length and stipe diameter of *G. pampeanus* selected strains cultivated on *Eucaliptus* sp. and *Populus* sp. sawdust.

Treatment	Basidioma weight (g)	Pileus diam. (cm)	Stipe length (cm)	Stipe diam. (cm)
ES + 751/12	21.89 ± 11.67a	5.18 ± 1.00a	7.57 ± 1.95a	1.43 ± 0.36a
ES + 748/12	23.06 ± 6.30a	5.25 ± 0.80a	10.99 ± 1.88a	1.52 ± 0.18a
ES + 47/99	28.37 ± 11.21a	5.23 ± 1.07a	10.36 ± 2.40a	1.52 ± 0.28a
PS + 751/12	26.11 ± 4.87a	4.71 ± 0.87a	8.37 ± 1.04a	1.46 ± 0.14a
PS + 748/12	29.31 ± 7.35a	4.92 ± 0.64a	10.79 ± 1.60a	1.63 ± 0.35a
PS + 47/99	20.72 ± 8.94a	4.72 ± 1.14a	8.07 ± 1.26a	1.29 ± 0.14a

ES: *Eucalyptus* sp. sawdust; PS *Populus* sp. sawdust; values obtained are means of all basidiomata harvested in each treatment ± SD. Values with the same letter in the same column have no significant difference ($P = 0.005$) according to Tukey's HSD test.

Means of pileus diameter varied from 4.71 to 5.25 cm (Table 2). No significant differences were found among the treatments. Regarding stipe measurements, we did not find significant differences among treatments and strains. Stipes varied from 7.5 to 10.9 cm long and 1.3 to 1.6 cm diam.

3.4. Biodegradation of substrates during cultivation process

The analysis of biodegradation of substrates was done when cropping period was finished (4.5 months after spawning). *G. pampeanus* had a strong capacity to degrade ES and PS (Table 3) and decreased the percentage of organic matter in 82.05% and 83.90%, respectively. Ashes content increased from 0.37 in untreated ES to 4.73% and from 1.28 to 8.53% in PS. Cellulose content decreased in both substrates from 66.65 to 60.25% in ES and from 63.54 to 54.46% in PS. On the other hand, hemicellulose increased for ES 23.6% but decreased for PSP 41.04%. Lignin content decreased in both treated substrates from 19.65 to 10.16% in ES and from 17.14 to 11.25% in PS.

3.5. Discussion

3.5.1. Determination of linear growth rates

When growth rate at 25 °C was evaluated, no significant differences in the values were found (data not shown). This could probably be due to the area where specimens where collected. Basidiomata were found in a region named "Región pampeana" where environmental conditions are distinctive and strains are probably genetically adapted to them. In general, worldwide cultivated species, such as *Agaricus bisporus*, *Pleurotus ostreatus*, and *Agrocybe cylindracea* have an optimal temperature of vegetative growth at 25 °C (Stamets, 1993) this is why we chose a range of 20–30 °C to evaluate the optimal temperature of this species. In fungi, the growth rate is a trait frequently used to estimate the ability of a strain to colonize a substrate, spread a new niche or exploit a newly contaminated environment (Plaza et al., 1998). Temperature for optimal mycelia growth is useful for spawn production and to optimize the incubation phase.

3.5.2. Determination of light requirements for primordia induction

Photoperiod is not necessary to induce the primordium formation but it is needed for fruiting body production. A publication by Kaufert (1936) seems to be the first indication that *Pleurotus* species required light. However, the most cultivated mushroom (*A. bisporus)* produces primordia in the darkness (Kurtzman and Martinez-Carrera, 2013). Sakamoto et al. (2007) observed that formation of *Flammulina velutipes's* fruiting bodies can be induced in complete darkness but under these conditions, fruiting bodies formed elongate stipe, had a poorly developed pileus and lacked a hymenium, suggesting that they cannot mature in complete darkness. However, after light treatment of the pinhead fruiting body, a pileus develops normally immediately, and the stipe also thickens and becomes increasingly pigmented. Aschan-Aberg showed that blue light was required for normal basidiomata growth of *F. velutipes* (Aschan-Aberg, 1960). Blue light between 400 and 480 nm was also required for some *Coprinus* species (Carlile, 1965). Recent advances in fungal photobiology using molecular tools and genomic analysis have shown specific phytochromes, photoreceptor proteins, transcription factors, light-regulated genes, and to a certain extent common regulatory pathways leading to mushrooms development and spore viability. It seems likely that all mushrooms, which require light, use a common regulatory pathway for basidioma development (Kurtzman and Martinez-Carrera, 2013). The formation of primordial for *G. spectabilis* in the darkness can be a useful sign. It can be considered as a biological indicator that mushroom metabolism is ready for fruiting, showing to the mushroom growers the end of the incubation phase. Thus bags can be removed and blocks can be transferred to the production room.

3.5.3. Production assay

All assayed strains obtained higher yield on PS which indicates a preference of this species for this substrate in culture. This fact is remarkable since this mushroom is always found in nature growing on ES and never on PS and both trees species are present in the "Region Pampeana".

As pointed out above, the highest BE was obtained with strain ICFC 748/12 on PS reaching a mean of 70.67%. Other xylophagous naturally occurring species which were also evaluated for mushroom production produced lower yields in non-supplemented sawdust. Uhart et al. (2008) has reported for *A. cylindracea*, BEs ranges from 56.5 to 59.4%. Omarini et al. (2009) has studied the production of *P. tenuiculus* in wheat straw and willow sawdust, obtaining BEs % from 40 to 53, respectively.

Wheat straw is generally a very good substrate for mushroom cultivation, and it has been used to cultivate *P. ostreatus, Pleurotus pulmonarius*, *Pleurotus djamor* (Ruán-Soto et al., 2006; Lechner et al., 2004) *Pleurotus eryngii*, *P. ostreatus, Volvariella volvacea*

Table 3
Percentages of dry matter, ashes, cellulose, hemicellulose and lignin content of two different substrates at the end of *G. pampeanus* production process.

	Organic matter (%)	Ashes (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
ES untreated	89.55 ± 0.10a	0.37 ± 0.06a	66.65 ± 0.29a	9.72 ± 0.28a	19.65 ± 0.24a
ES spent substrate	16.08 ± 0.21b	4.73 ± 0.02b	60.25 ± 0.26b	12.72 ± 0.14b	10.16 ± 0.44b
PS untreated	88.84 ± 0.21a	1.28 ± 0.01c	63.54 ± 0.61c	13.67 ± 0.27b	17.14 ± 0.07c
PS spend substrate	14.31 ± 0.15b	8.53 ± 0.02d	54.46 ± 0.51d	8.06 ± 0.31c	11.28 ± 0.05d

ES: *Eucalyptus* sp. sawdust. PS: *Populus* sp. sawdust. Strain used ICFC 748/12. Values with the same letter in the same column have no significant difference ($P = 0.05$) according to Tukey's HSD test.

(Philippoussis et al., 2001), *A. cylindracea* (=*Agrocybe aegerita*) (Philippoussis et al., 2001; Uhart et al., 2008), and *L. edodes* (Gaitán-Hernández et al., 2006) among others. However, we observed that wheat straw did not result in an adequate substrate for *G. pampeanus*. We made some assays on wheat straw but bags were systematically contaminated and never finished the incubation phase (data not shown). On the other hand, only 5–8% of the bags with sawdust were contaminated during the spawn running. This is probably due to the lower colonization capacity that these strains had and the long time required for finishing the incubation phase. While *G. pampeanus* require 75 days of spawn running time, other species need less time. *P. ostreatus* requires, for example, 21 to 30 days for this phase (Lechner and Albertó, 2011) and *A. bisporus* (Stamets, 1993) required 12–15 days. Production time lasted about two months in which basidiomata were harvested. A flushes patter was not observed since basidiomata fruited on the blocks non-synchronously.

Means of pileus diam. varied from 4.71 to 5.25 cm. The values obtained in culture were lower than those found in nature. When specimens of *G. pampeanus* were collected in the field, we observed that basidiomata were quite larger, reaching 10–12 cm diam. In our opinion, the smaller diameter of basidiomata could be due to the production technique we have developed. We are currently carrying out an experiment on *Eucalyptus* logs, using the same strains and we harvested basidiomata from 6.60 to 9.3 cm diam. (data not shown).

As regards stipe measurements, they varied from 7.5 to 10.9 cm long and 1.3 to 1.6 cm diam. No significant differences were found between treatments. Recently Lechner and Albertó (2011), studied basidiomata's morphological traits of fourteen naturally occurring Pleurotus strains; they also proved that stipe length did not vary when they were cultivated in three different substrates. The length of stipe is an important factor for mushroom farmers because when stipes are too long they need to be cut and discarded. As a consequence, shorter stipes are preferred.

3.5.4. Biodegradation of substrates during cultivation process

The analysis of biodegradation of substrates was done when cropping period was finished showing that *G. pampeanus* had a strong capacity to degrade sawdust and decrease the percentage of organic matter. When ashes content was compared between treated and untreated sawdust, a strong increse in ashes content of treated substrate was observed reaching 1278% for ES and 666% for PS. The increase of ashes, considered as mineral residue of incinerated organic matter, could be explained by two factors: (i) *exogenous supply of minerals through watering* since blocks were exposed to tap water which is rich in minerals and salts; (ii) *fictitious relative increase of minerals*. The latter due to the increase of minerals that fungal biomass contains and concentrates as part of its metabolism. After 4.5 months of growing, mushroom degraded an important amount of organic matter. From the total amount of energy obtained from the substrate, one part is used to produce mycelia and the rest is lost as CO_2 and H_2O during cellular respiration process. As a consequence, when a mass of treated substrate is weighted for the ashes analysis, it contains part of the degraded sawdust and part of fungal biomass. The latter accumulate minerals which are originally in sawdust but which are finally obtained during fermentation process by fungal metabolism. The same results are found in many other studies. Rajarathnam et al. (1997) has mentioned that a relative increase in the ash content was observed in the course of degradation of the substrate and utilization of its components in shiitake strains. Chantaraj (2000) and Sánchez et al. (2002) proposed that an increase in mineral content is one of the changes that substrates undergo during enzymatic breakdown and the resulting organic matter is utilized while the fungi are in the vegetative development stage.

Cellulose content decreased in both substrates 9.6% for ES and 14.3% for *Populus* sawdust. On the other hand, hemicellolose increased for ES 23.6% but decreased for PS 41.04%. Lignin content decreased in both treated substrates 48.3% for ES and 34.18% for PS. This values were lower compared with those obtained with other species. Nazareth and Sampy (2003) reported that the decrease in lignin and cellulose content of hardwood sawdust by *Panus tigrinus* (=*L. tigrinus*) after a 4-month incubation period was 56% and 64%, respectively, while, in Lechner and Papinutti (2006), the decrease of lignin and cellulose content in wheat straw was 21.49% and 53.26%, respectively, in a similar period of time for the same species.

We observed that when *G. pampeanus* was grown on PS, the holocellulose content drastically diminished (55%) showing the preference of the mushroom for this compound in this substrate. On the other hand, for ES, the holocellulose increased (13.98%) but lignin content decreased 48.3%; thus results showed that when *G. pampeanus* grows on *Populus* it prefers to degrade holocellulose, meanwhile when it grows on *Eucalyptus*, it prefers to degrade lignin. Lignin breakdown by white rot fungi is performed by a complex panel of oxidizes such as phenol oxidase, the production and activity of all these enzymes is physiologically coordinated (Cullen and Kersten, 2004).

Similar results have been found in shiitake (*L. edodes*) growing on lignocellulosic residues. Gaitán-Hernández et al. (2011) mentioned that when shiitake grows on lignocellulose substrates, cellulose degradation is lower than that of lignin. Lignin is continuously broken down by shiitake during the primordium formation stage with greater values than that of cellulose. The lignin degradation capacity of shiitake was different in substrates such as cereal straw and vineyard prunings, mainly because of differences in the composition of the primary polymers in the lignin complex. This probably affected the affinity of the mushrooms' enzymes for the substrates. Morais et al. (2000), also pointed out that the activity of these enzymes depends on the composition of the substrate and enviromnental condition. The variability in the degradation capacity of lignocellulosic components in the substrates is influenced not only by the substrate's nature, but also by environmental factors and, most importantly, by genetic factors among species or even among strains of the same species (Omarini et al., 2009). Although, in both substrates the mushroom decayed the organic matter similarly, BE were significantly higher in PS, indicating the fungus can absorb the nutrients better and produce higher biomass. In this work we observed that strain selection is relevant to manage high yields since only one of the three strains assayed produced significantly higher BE.

Few works have described cultural characters or the type of rot caused by *Gymnopilus* species. Murrill (1940) mentioned that the type specimen of *G. armillatus* Murrill was found causing a whitish decay in a living red gum tree (*Liquidambar styraciflua* L.). Gilbertson (1980) described cultural features of *Gymnopilus sapineus* (Fr.) Maire and recorded it as a brown-rot fungus. Fausto-Guerra et al., 2002 observed that many *Gymnopilus* strains could grow on lignin media and give positive results on phenol oxidase reactions. These results indicate that *Gymnopilus* decomposes cellulose but also has the ability to decay lignin. For this reason the genus has been reported both as of white and brown-rot. Sede and López (1999) described a strain of *G. spectabilis* var. *pampeanus* (*Speg.*) Singer [=*G. pampeanus* (Speg.) Singer], obtained from a standing *Eucalyptus* in Argentine, based on Noble's method (Nobles, 1965). They observed positive results for oxidase reactions (gallic acid +++; tanic acid +++; tyrosine ++; paracresol +++; guaiacol −). Based on the results of degradation obtained in this work, we support the idea of considering *G. pampeanus* as white rot fungi.

In conclusion, it was possible to determine some optimal conditions for the production *G. pampeanus* being this the first report of the cultivation of this mushroom on sawdust wastes. The optimal

spawn running temperature was 25 °C; highest yield was obtained with strain ICFC 748/12 on PS with BE of 70, 88%, which is similar to values obtained for other naturally occurring mushroom on un-supplemented sawdust. Primordia can be induced without the presence of light, but photoperiod is necessary for the normal development of basidiomas. Although it is common to see *G. pampeanus* in nature growing on the base of *Eucalyptus* trees, the most suitable substrate shown in this work has resulted PS. We observed that *G. pampeanus* has a strong capacity to degrade ES and PS decreasing the percentage of organic matter. *G. pampeanus* decomposes cellulose but also has the ability to decay lignin thus we support the idea that *G. pampeanus* is a withe rot fungi. Apart from reducing on the environment the impact of the wastes, this bioconversion process represents an economically sound strategy to convert agroresidues into nutritional food.

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